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Plant hosts and parasitoid associations of leaf mining flies (Diptera: Agromyzidae) in the Canberra region of Australia

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Abstract Many leaf mining flies (Diptera: Agromyzidae) are important economic pests of agricultural crops and ornamental plants, and species-rich hymenopteran parasitoid complexes are important in their control. Australian agromyzids are poorly studied, and little is known about their host plants, ecology or natural enemies. We surveyed native and naturalised species of leaf mining flies in Tallaganda National Park, New South Wales and the Australian Capital Territory. Malaise and emergence trapping in Tallaganda yielded 70 agromyzid specimens from six species in four genera: *Cerodontha* Rondani, *Liriomyza* Mik, *Phytoliriomyza* Hendel and *Phytomyza* Fallen. Of the six species collected, three are Australasian species, two are naturalised species introduced from Europe and one could not be determined to species. The Australian *Cerodontha* (*Cerodontha*) *milleri* Spencer represented most of the individuals caught in both Malaise and emergence traps. A total of 163 agromyzid and 98 parasitic wasp specimens were reared from plant samples with agromyzid mines in the Canberra region. Most agromyzids and parasitoids were reared from the weed *Sonchus oleraceus* L. (Asteraceae). All the agromyzids reared belonged to two introduced species of the genera *Phytomyza* and *Chromatomyia* Hardy. The biodiversity of parasitic wasps reared was high with 14 species from seven genera and three families. *Hemiptarsenus varicornis* (Girault) (Eulophidae), a widespread Old World agromyzid parasitoid, was the most numerous parasitoid reared in our survey.

Key words *Cerodontha milleri*, *Chromatomyia*, *Hemiptarsenus varicornis*, *Phytomyza*, *Sonchus oleraceus*.

Introduction

Agromyzid flies are small (2–6 mm) insects whose larvae feed entirely in living plant tissues, primarily as leafminers but also in stems, roots and seeds. A number of agromyzids are important economic pests of agricultural crops and ornamental plants in many countries around the world (Spencer 1973; Dempewolf 2006; Scheffer *et al.* 2006). Most major pest agromyzid species have been spread inadvertently to new locations beyond their original geographical range, coincident with the increase in global trade that has taken place over the past half century (Spencer 1973; Minkenberg 1988; Dempewolf 2006). This is especially true of several polyphagous pest species, including *Liriomyza huidobrensis* (Blanchard), *L. trifolii* (Burgess) and *L. sativae* Blanchard. Introduced populations of these pest leafminers often result in outbreaks leading to substantial losses and sometimes crop failure (Spencer 1973; Shepard *et al.* 1998).

Agromyzids are considered difficult insects to work with taxonomically because of their small size and general uniformity in external morphology. Closely related species are often difficult to distinguish and may occur together on the same host plants (Kulp 1968; Spencer 1990; Scheffer & Wiegmann 2000; Dempewolf 2006). In most regions of the world, both native and naturalised agromyzid faunas have been poorly studied, and, of the species known to be present, little is known about their host plants or ecology (Gratton & Welter 2001). This is especially true in Australia, where few surveys of agromyzids have been conducted, and host plant associations have been determined for only 44 of the 150 known species (Spencer 1977). Given the recent spread of invasive and polyphagous pest leafminers to south-east Asia and New Zealand (Shepard *et al.* 1998; Andersen *et al.* 2002; Scheffer *et al.* 2006), an improved understanding of the current Australian agromyzid fauna is essential.

Agromyzids are typically attacked as eggs, larvae or pupae by numerous parasitoid wasps in as many as 10 hymenopteran families (Spencer 1973). The species-rich hymenopteran parasitoid complexes associated with leaf mining flies are of great importance in controlling invasive agromyzids (Johnson 1993; Murphy & La Salle 1999). However, there are very limited records of agromyzid parasitoids in Australia (Belokobylskij *et al.* 2004; Edwards & La Salle 2004). In this study we used

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Table 1 Trap locations in Tallaganda National Park

Emergence trap	Malaise trap
1: 35°24′48.8″S, 149°32′11.9″E	1: 35°24′51.2″S, 149°32′09.4″E
2: 35°24′50.6″S, 149°32′09.4″E	2: 35°24′48.3″S, 149°32′12.4″E
3: 35°24′52.7″S, 149°32′06.9″E	3: 35°24′52.8″S, 149°32′06.9″E
4: 35°24′53.1″S, 149°32′07.5″E	4: 35°24′53.4″S, 149°32′07.2″E
5: 35°24′53.1″S, 149°32′07.5″E	5: 35°24′53.0″S, 149°32′08.2″E

several trapping and rearing techniques to survey native and naturalised species of leaf mining flies in the Canberra region to establish plant host–agromyzid and agromyzid–parasitoid associations.

METHODS

Study sites

The study was conducted in three discrete locations: Tallaganda National Park, New South Wales, a nearby roadside creek at Forbes Creek (35°26′S, 149°31′E), and in Canberra, Australian Capital Territory at Black Mountain Reserve and several urban locations. Tallaganda National Park lies 7 km east of Hoskinstown, New South Wales. The Tallaganda site (35°24′S, 149°32′E) is a small grassy meadow, surrounded by wet sclerophyll forest on South Black Range, at an altitude of 1129 m–1165 m. The Black Mountain site backs onto the CSIRO laboratories (35°16′S, 149°07′E) and is a reserve of native vegetation. The urban sites were taken from a variety of habitats including a backyard in the suburb of Evatt (35°12′S, 149°04′E) with native and introduced garden plants as well as weed species and a site on the slopes of Mt Rogers in the suburb of Flynn (35°11′S, 149°03′E).

Malaise and emergence traps

Five Malaise and five emergence traps were used to collect Agromyzidae at Tallaganda (Table 1). Traps were constructed of fine mesh and collected insects that moved upwards and fell into sample bottles filled with 95% ethanol. Malaise traps (Fig. 1a) were placed across insect flight paths around the edges of the meadow because edge habitats are known to have higher numbers of Agromyzidae (Hagvar *et al.* 1994). The emergence traps (Fig. 1b) enclosed all plant species in approximately 0.56 m^2 of ground cover (Stephens 2005), and were placed to sample the majority of plant species in the meadow. Sampling was undertaken from 30 November 2005 to 24 January 2006, corresponding to the seasonal peak of Agromyzidae infestations in summer (e.g. Johnson *et al.* 1980; Chen *et al.* 2003). Bottles were collected and replaced regularly creating four sample periods: 30 November–6 December 2005 (I), 6–21 December 2005 (II), 21 December 2005–9 January 2006 (III) and 9–24 January 2006 (IV). Samples were sorted using a stereomicroscope and all Agromyzidae were removed.

Collection of plant host material

Plant material was collected from December 2005 to January 2006 at all sites and focused on likely agromyzid plant hosts such as grasses, herbs, forbs, sedges and small shrubs (Spencer 1977). Plants were examined for agromyzid leaf mines or similar damage, and several samples including damaged leaves were then collected.

Rearing adult flies and parasitoids

Mines were examined under a stereomicroscope and dissected, when pupae were visible, by breaking the surrounding epidermal leaf tissue and lifting the pupae into small vials with a dry fine-haired brush. A similar method was used by Frost (1924), but Frost dried the pupae for several hours before placement in air-tight vials. We sealed the vials with cotton wool to allow movement of air and moisture and eliminated the drying step. Individual vials contained either a single or multiple pupae, and vials were grouped into larger air-tight transparent containers. Wet tissue paper was added to these larger containers for several days, and removed when condensation was observed inside the vials.

Another two types of rearing chambers were used to rear pupae and larvae left in mines in collected plant material. Rigid transparent plastic chambers ($23 \times 17 \times 18$ cm), with mesh at one end and an opening for the hand at the other, were used to house each separate sample of collected plant material (Fig. 2b). Plant stems or midribs were wrapped in wet tissue paper and placed in small containers of water to maintain turgidity of the leaves. The second method used transparent air-tight plastic zip-lock bags (23×30 cm) to house the plant samples. Thick tissue paper was added to absorb moisture due to condensation and was replaced periodically to prevent excess condensation and mould. Chambers, vials and plastic bags were inspected every few days for the emergence of adult wasps and flies. Any adult wasps and flies that emerged were removed and placed directly into 95% ethanol. Agromyzids either were stored in ethanol for molecular studies or were dehydrated using a critical point drier and then pinned. All parasitic wasps were dried and pinned. All specimens are stored in the Australian National Insect Collection (ANIC) at CSIRO Entomology in Canberra.

Agromyzids were identified using morphological keys (Spencer 1977; Dempewolf 2006), coupled with the dissection of male genitalia. Specimens were also compared with identified specimens held in ANIC.

RESULTS AND DISCUSSION

Malaise and emergence trapping

Malaise and emergence trapping in Tallaganda yielded 70 Agromyzidae specimens from six species in four genera (Table 2), all within the subfamily Phytomyzinae. Five of the species could be fully identified, but the single *Liriomyza* Mik female could not be identified further because, at this time, female *Liriomyza* cannot be reliably identified using available keys. Four of the recovered species are Australasian, while *Phytomyza vitalbae* Kaltenbach was introduced into Australia

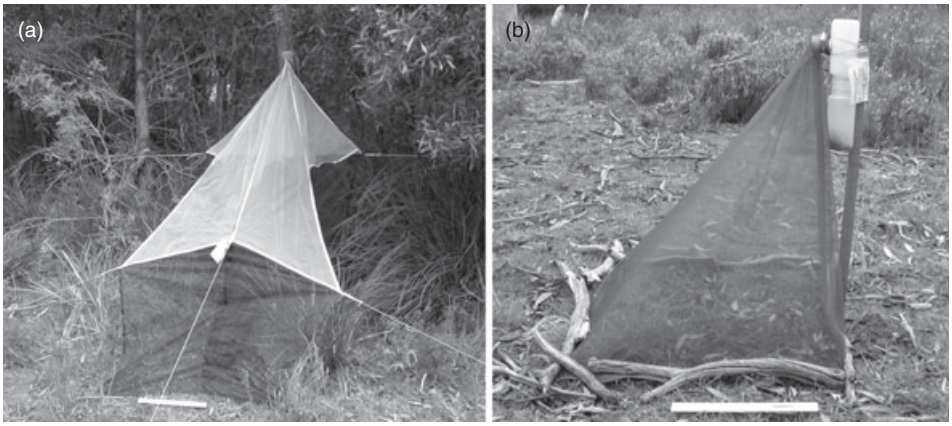

Fig. 1. Trapping methods. (a) Malaise trap; (b) emergence trap. A 30 cm ruler included.

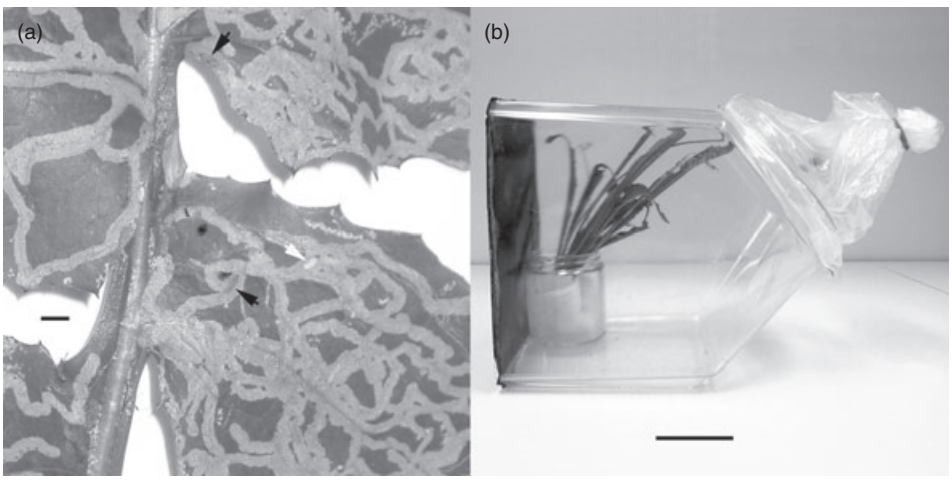

Fig. 2. Rearing methods. (a) *Sonchus oleraceus* with mines containing pupae and larvae, from Canberra urban area. Arrows indicate pupae within mines: black arrow, parasitised; white arrow, not parasitised. Scale line = 5 mm. (b) Rigid plastic rearing chamber. Scale line = 5 cm.

Table 2 Species of Agromyzidae collected at Tallaganda National Park from Malaise and emergence traps

Agromyzid species	*n*	Male : female	Malaise : emergence
Cerodontha (*Cerodontha*) *milleri* Spencer	60	30:30	42:18
Cerodontha (*Cerodontha*) *robusta* Malloch	1	0:1	1:0
Cerodontha (Icteromyza) triplicata (Spencer)	2	2:0	2:0
Liriomyza sp.	1	0:1	1:0
Phytoliriomyza tricolor (Malloch)	4	3:1	3:1
Phytomyza vitalbae Kaltenbach	2	0:2	2:0

from Europe and is naturalised (Spencer 1977). *Cerodontha* (*Cerodontha*) *milleri* Spencer represented 86% of the individuals caught.

All six species of Agromyzidae from Tallaganda were found in Malaise trap samples (Table 2). Malaise traps collected 82% of trapped agromyzids, with most caught in period I (30 November–6 December 2005), and none collected in period IV (9–24 January 2006) (Fig. 3a).

Nineteen agromyzids were collected from emergence traps in Tallaganda, including 18 specimens of *Cer. milleri* and a single *Phytoliriomyza tricolor* (Malloch) (Table 2). Eighteen of the 19 agromyzids were collected from emergence traps 1 and 2. Angiosperms enclosed by these traps were removed and identified to develop a list of possible host plants. The grass, *Anthoxanthum odouratum* L. (Poaceae), was found in both traps and dominated the vegetation in emergence trap 2. Fourteen *Cer. milleri* were collected from emergence trap 2, while only three were collected from emergence trap 1 which was dominated by a *Persoonia* Sm. species (Proteaceae).

Malaise trapping captured more species and more specimens than emergence trapping on all dates. Although emergence traps compromise broad scale environmental collection of agromyzids, they suggest putative agromyzid host plant species in the absence of distinct mines bearing larvae and pupae. *Cerodontha milleri* dominated the agromyzid assemblages collected from Malaise and emergence traps. Surveys of the plants within the emergence traps revealed that *Cer. milleri* abundance paralleled that of *An. odouratum*, an introduced pasture grass belonging to a family known to host other *Cerodontha* Rondani larvae (Spencer 1977; 1990; Scheirs

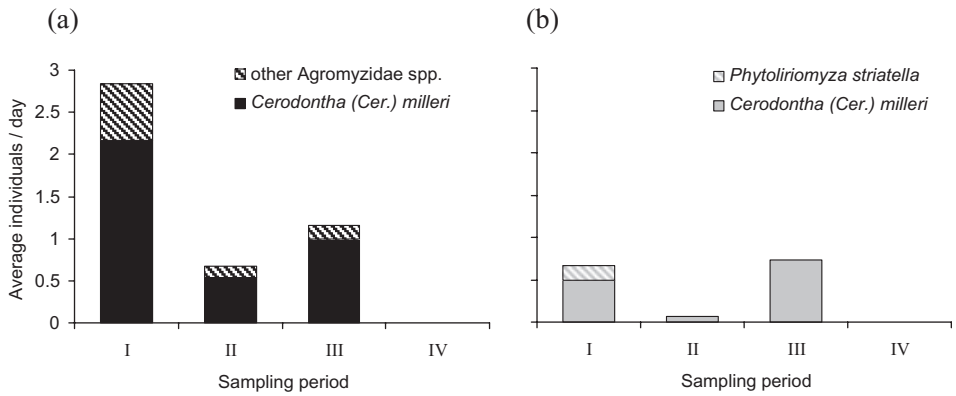

Fig. 3. Average number of Agromyzidae collected per day in each sampling period showing *Cerodontha* (*Cerodontha*) *milleri* and other species in (a) Malaise traps, and (b) emergence traps. Emergence trap 5 was removed in sampling period IV because of damage. (I) 30 November–6 December 2005, $n = 6$ days; (II) 6–21 December 2005, $n = 15$ days; (III) 21 December 2005–9 January 2006, $n = 19$ days; and (IV) 9–24 January 2006, $n = 15$ days.

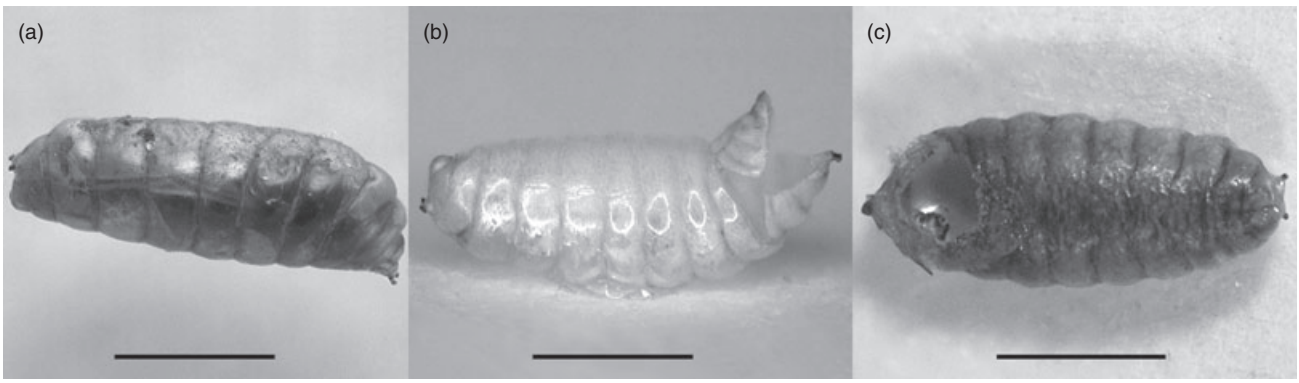

Fig. 4. Agromyzidae pupae removed from *Sonchus oleraceus* containing (a) a parasitic wasp, *Trigonogastrella parasitica*. Pupae showing emergence holes by (b) an agromyzid, lateral view; and (c) parasitic wasp *Opius* sp. 2, dorsal view. Scale lines = 1 mm.

et al. 1996). *Anthoxanthum odouratum* also had damaged leaf blades which may have been female feeding scars (Eber *et al.* 2001). However, emergence traps do not provide definitive evidence for a leafminer–host plant association. Future survey work will specifically target *An. odouratum* to determine whether it is indeed the host of *Cer. milleri*. Because *An. odouratum* was originally introduced into Australia from Eurasia (Csurhes & Edwards 1998), if it is found to host *Cer. milleri*, the native status of both *Cer. milleri* and its close relative *Cer.* (*Cer.*) *australis* Malloch might need to be reassessed. However, the use of an introduced plant by a native leafminer could also represent a recent dietary shift or expansion from a native Australian host plant.

Persoonia may be the plant host for *Phytol. tricolor* as both were only found in emergence trap 1, which was dominated by a *Persoonia* sp. The *Persoonia* leaves did appear to have leafmines, but no flies or parasitoids were reared. Some agromyzid species, such as *Chromatomyia suikazurae* Sasakawa, have a mid-spring peak in abundance (Kato 1994). We surveyed the Tallaganda site in late spring through summer and may have missed the peak abundance of *Phytol. tricolor* at Tallaganda, collecting only one adult in the first sampling period. Given that the first trapping period collected the greatest abundance of agromyzids strongly suggests that earlier trapping would have caught additional specimens and possibly additional species.

Rearing of leafmining flies and hymenopteran parasitoids

In the rigid rearing chambers (Fig. 2b) plant material dried out rapidly, and few adult insects emerged. In the plastic bags with high humidity, insects emerged even after plant material decomposed substantially. For pupae that had been removed from the plant material, pupal deaths appeared to be higher in vials with larger numbers of pupae per vial. Of the 129 pupae removed from leafmines on *Sonchus oleraceus* L. (Asteraceae), adults failed to emerge from 44 (34%). Some of the dead pupae clearly contained wasps (Fig. 4a), but it was usually difficult to distinguish between unemerged flies and wasps. However, pupae from which wasps and flies have emerged can be distinguished as the flies break open the puparium laterally (Fig. 4b) and wasps emerge through a circular hole that they chew on the dorsal surface of the puparium

Table 3 Agromyzidae and parasitoid species reared from Forbes Creek, Black Mountain and Canberra Urban areas

Plant host	Agromyzidae		Parasitoids		
Species	Species	*n*†	Family	Species	*n*†
Sonchus oleraceous L. (Asteraceae)	*Chromatomyia syngenesiae* Hardy	132	Pteromalidae	*Trigonogastrella parasitica* Girault	10
			Eulophidae	*Hemiptarsenus varicornis* (Girault)	10
			Eulophidae	*Closterocerus mirabilis* Edwards & La Salle	1
			Eulophidae	*Asecodes* species	2
			Braconidae	*Opius* sp. 1	7
			Braconidae	*Opius* sp. 2	9
			Braconidae	*Opius* sp. 3	3
			Braconidae	*Opius* sp. 4	3
			Braconidae	*Opius* sp. 5	2
			Braconidae	*Opius* sp. 6	2
			Braconidae	Braconidae sp.	1
Plantago lanceolata L. (Plantaginaceae)	*Phytomyza plantaginis* Goureau	26	Eulophidae	*Diglyphus isaea* (Walker)	9
			Pteromalidae	Pteromalidae sp.	3
			Eulophidae	*Neochrysocharis* sp.	6
Unknown plants	*Chromatomyia syngenesiae*	5	Pteromalidae	*Trigonogastrella parasitica*	5
			Pteromalidae	Pteromalidae sp.	3
			Eulophidae	*Hemiptarsenus varicornis*	12
			Eulophidae	*Neochrysocharis* sp.	1
			Braconidae	*Opius* sp. 1	1
Argyranthemum frutescens ssp. *foeniculum* (Asteraceae)			Pteromalidae	*Trigonogastrella parasitica*	7

†Total (*n*) from all rearing methods.

(Fig. 4c). Most agromyzids and parasitoids emerged within 10 days of plant collection, but some emerged up to 21 days after collection.

Active agromyzid mines on plants were not discovered at the Tallaganda site, and therefore no agromyzids or parasitoids were reared. The only leafmines observed occurred on a species of *Persoonia*, but the mines on this plant were apparently empty, as numerous parasitoid emergence holes were evident, and attempts to rear leafminers were unsuccessful.

In contrast to the Tallaganda site, agromyzid mines were conspicuous on several plant species in urban areas. A total of 163 agromyzid and 98 parasitic wasp specimens were reared from three identified and several unidentified plant species from Forbes Creek and Canberra (Table 3). Two agromyzid species in Phytomyzinae were reared in this survey, *Phytomyza plantaginis* Goureau and *Chromatomyia syngenesiae* (Hardy), which are both considered to be introductions from Europe (Spencer 1977). *Phytomyza plantaginis* was only reared from *Plantago lanceolata* L. (Plantaginaceae), and only females were recovered. *Chromatomyia syngenesiae* was reared from several host plants, with most being reared from *S. oleraceus*.

A total of 14 species of parasitic wasps from seven genera and three families were reared from mines (Table 3). All the wasps reared in this survey belong to families having species known to parasitise agromyzids (Spencer 1973; Neuenschwander *et al.* 1987; Murphy & La Salle 1999; Bjorksten *et al.* 2005). *Hemiptarsenus varicornis* (Girault) (Eulophidae) and *Trigonogastrella parasitica* Girault (Pteromalidae) were the most numerous parasitoid species. *Hemiptarsenus varicornis* parasitised both *Phytom. plantaginis* and *Chrom. syngenesiae*, whereas *T. parasitica* was only reared from *Chrom. syngenesiae*. All the wasp species other than *Diglyphus isaea* (Walker) (Eulophidae) parasitised *Chrom. syngenesiae,* whereas only three wasp species parasitised *Phytom. plantaginis*. A total of six *Opius* species (Braconidae) were reared from the samples, and together accounted for over half the parasitisation on the *Chrom. syngenesiae* on *S. oleraceus*. Belokobylskij *et al.* (2004) provided a review of *Opius* species that attack agromyzids in Australia, but at that time there were only three species that had been reliably reared from these hosts. Clearly, further studies on the taxonomy of these wasps are necessary.

Rearing methods are superior to trapping methods for determining parasitoid–agromyzid and plant–agromyzid relationships. All rearing techniques employed in this survey were successful; however, the removal of pupae into vials had logistical advantages and produced data with more certainty. In the large rigid plastic chambers, plant material dried out within a couple of days, probably killing any larval stages present, and it was difficult to observe and collect mobile adults. Plastic bag chambers prevented plant material from drying out, and adults continued to emerge in these chambers even when plant material became mouldy. We recommend plastic bag chambers for rearing larvae until pupation and subsequent transfer of pupae to separate vials. Vials were more compact and did not dry out pupae or become mouldy. However, multiple pupae in vials suffered high mortality, as overcrowding prevented adult emergence from numerous pupae in close contact.

The method of removing pupae from plant material and rearing in vials avoids the confusion of parasitoid complexes in plants hosting more than one agromyzid species, a common phenomenon in agromyzids (Spencer 1990). However, Gratton and Welter (2001) found removal of *Calycomyza platyptera*

(Thomson), which pupate within mines, unsuccessful as pupae dried out. We prevented pupae from drying out by keeping the vials in an air-tight container and adding wet tissue paper for a short period. Pupal removal also provided additional information such as the differing emergence holes of flies and wasps from fly pupae (Fig. 4b,c).

From the rearing portion of our study, we reared two introduced leafminers, *Chrom. syngenesiae* and *Phytom. plantaginis*. Both species were reared from introduced host plants upon which each of the reared leafminers is known to feed (Spencer 1990). *Chromatomyia syngenesiae* is an oligophagous leafminer, which feeds almost exclusively on species of Asteraceae with the exception of two documented cases on Apiaceae and Fabaceae (Spencer 1990). *Phytomyza plantaginis* feeds exclusively on species of *Plantago* L. in the Plantaginaceae. Only female *Phytom. plantaginis* were reared in this study, which was also reported by Spencer (1977). A shift to parthenogenesis was noted in this species after introduction into North America where only females are known (Frick 1951; Spencer & Steyskal 1986), and it seems probable that Australian populations of *Phytom. plantaginis* are also parthenogenetic.

Significance for Australia

We found only eight agromyzid species in the Canberra region of Australia, which is a low species richness relative to results from surveys of both northern and southern hemisphere temperate regions (Scheirs *et al.* 1995; 1996; 1999; Salvo & Valladares 1999; Boucher & Wheeler 2001; SJ Scheffer in prep. 2007). Some of these surveys covered larger areas or were of longer duration than our study, which may account for some of the difference. However, our finding of a less diverse fauna is consistent with previous reports of reduced diversity in the Australian agromyzid fauna (Spencer 1977) and warrants further study. The fact that we only found species of Phytomyzinae is puzzling given that the diversity of Phytomyzinae and Agromyzinae in Australia are approximately the same (Spencer 1977). We suggest this is a result of small sample size in combination with searching specifically for leafminers, which are more likely to be phytomyzines than agromyzines, many of which feed in seeds, stems and roots.

Our results indicate that *S. oleraceus* supports a rich reservoir of agromyzid parasitoids in the Canberra region. Similarly, Chen *et al.* (2003) found that 11 of the 14 species of parasitoids detected in an area were attacking agromyzids on weed species, with *S. oleraceus* harbouring the highest level of parasitism of agromyzids.

Native parasitoids are suggested to be the most effective form of biological control for invasive agromyzids, as they rapidly begin to use the new invasive species as a host (see Murphy & La Salle 1999). Bjorksten *et al.* (2005) reported 100% parasitism of agromyzids on a beetroot crop by native parasitoids within 3 weeks. Similarly, native parasitoids have provided good control of invasive agromyzids in Senegal (Neuenschwander *et al.* 1987), Malaysia (Sivapragasam *et al.* 1999), Indonesia (Shepard *et al.* 1998) and Vietnam (Thang 1999).

The pest agromyzids, *L. huidobrensis* and *L. sativae*, have reached South-East Asia, and their arrival in Australia should be regarded as impending (Shepard *et al.* 1998; Bjorksten & Robinson 2005; Bjorksten *et al.* 2005). *Hemiptarsenus varicornis* is an important agromyzid parasitoid in Indonesia, comprising 92% of parasitoids on *L. huidobrensis* and 60% on *L. sativae* (Rauf *et al.* 2000). *Hemiptarsenus varicornis* was the most numerous parasitoid reared in our survey of the Canberra region. *Opius* spp. were also common in both studies. This implies that these native parasitoids should be considered for biological control before importing exotic parasitoids if the pest agromyzids, *L. huidobrensis* and *L. sativae*, reach Australia.

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